Clinical Specificity of the Enzyme Immunoassay Test for Coccidioidomycosis Varies According to the Reason for Its Performance

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The diagnosis of coccidioidomycosis relies heavily on serologic test results in addition to clinical history, physical examination, and radiographic findings. Use of the enzyme immunoassay (EIA) has increased because it is rapidly performed and does not require referral to a reference laboratory, as do complement fixation and immunodiffusion tests. However, interpretation of immunoglobulin M (IgM) reactivity by EIA in the absence of immunoglobulin G (IgG) reactivity has been problematic. We conducted a retrospective medical record review of all patients with such IgM reactivity at our institution to identify situations where the finding was more likely to be clinically specific for coccidioidal infection. From 1 January 2004 through 31 December 2008, a total of 1,117 patients had positive EIA coccidioidal serology or EIA IgM-only reactivity; of these, 102 patients (9%) had EIA IgM-only reactivity. Among the 102 patients with EIA IgM-only reactivity, 60 were tested to evaluate symptomatic illness, 13 for follow-up of previously abnormal serology, and 29 for screening purposes. Of the 102 patients, 80 (78%) had positive serologic findings by other methods or had positive culture or histology. Fifty-four (90%) of the 60 patients whose serology was performed to evaluate symptomatic illness had coccidioidal infection, whereas 13 (45%) of 29 patients whose serology was performed for screening purposes had coccidioidal infection. Of the 102 patients with isolated IgM reactivity by EIA, 12 later seroconverted to IgG and IgM reactivity. The use of EIA for screening in 29 asymptomatic persons was associated with unconfirmable results in 13 (45%). Although the majority of patients in our study with isolated IgM reactivity by EIA had probable or confirmed coccidioidomycosis, this result must be interpreted with caution for asymptomatic patients.

Coccidioidomycosis is a fungal infection endemic to the desert areas of the southwestern United States. Infection with this airborne fungus is asymptomatic in nearly two-thirds of infected persons; the remaining persons have a spectrum of primarily respiratory symptoms that often present with a flulike illness or as community-acquired pneumonia (1).

In addition to a careful history and physical examination, the evaluation of coccidioidomycosis relies heavily on serologic testing. While serologic tests for *Coccidioides* organisms are considered more reliable than for other fungal infections (2), the sensitivity of coccidioidal serologic testing ranges from 0% to 100%, depending on the ability to mount an antibody response to the infection, the presence of an immunocompromising illness or medication, and the timing of the blood draw relative to the onset of symptoms (3).

Coccidioidal serologic testing using an enzyme immunoassay (EIA) has been embraced in the area where coccidioidomycosis is endemic because of its ease of use and rapid turnaround time, whereas other serologic studies require sending the specimen to a reference laboratory. In addition, the EIA is more sensitive early in the disease process than are complement fixation (CF) and immunodiffusion (ID) (3, 4). However, the EIA has generated some controversy, especially as it pertains to the particular finding of immunoglobulin M (IgM) reactivity in the absence of any immunoglobulin G (IgG) detected (EIA IgM$^+$/IgG$^-$). The few publications on this subject have been mixed: 1 study showed no false-positive results on EIA IgM$^+$/IgG$^-$ for patients symptomatic for coccidioidomycosis (5), another demonstrated 2.2% (6), and a third study demonstrated an 82% false-positive rate (7). The finding of IgM reactivity in the absence of IgG by EIA is therefore

difficult to interpret. The aim of this study was to further characterize the laboratory finding of IgM-only reactivity by EIA to clarify situations where the finding is more likely to be clinically specific for coccidioidal infection.

MATERIALS AND METHODS

Patients were identified by reviewing the records of all patients with reactive coccidioidal serology performed at our institution from 1 January 2004 through 31 December 2008. All patients with an IgM-only EIA reactivity (EIA IgM$^+$/IgG$^-$ result) were compiled into a data set. The records of such patients were reviewed for demographics, symptoms at the time of the serologic testing, reason for the serologic test (evaluation of symptoms, screening, or follow-up on previously abnormal serology), comorbid illnesses, and details of the coccidioidal illness (if present, including symptoms, laboratory studies, results of radiographs, microbiology, histology, treatment, and outcome). This study was approved by the Mayo Clinic Institutional Review Board.

The strength (or likelihood) of diagnosis of coccidioidomycosis was described along a continuum as follows. (i) Confirmed coccidioidomycosis required the identification of spherules in cytology or histologic specimens or growth of *Coccidioides* species in culture. (ii) Highly probable coccidioidomycosis required the presence of coccidioidomycosis-com-

patible symptoms, typical radiographic abnormalities, and positive serology. (iii) Probable coccidioidomycosis required either compatible symptoms or radiograph findings in the presence of positive serology (EIA IgG$^+$, ID IgM$^+$, and/or IgG$^+$, or CF titer of $\geq$1:2). (iv) Possible coccidioidomycosis was diagnosed when IgM-only EIA reactivity was not supported by other serologic coccidioidal results but was still accompanied by either compatible symptoms or radiographic findings. (v) Unconfirmed coccidioidomycosis was identified when the EIA IgM$^+$/IgG$^-$ was the sole abnormality, without any supportive serology, symptoms, or radiographic abnormalities.

"Typical symptoms" of coccidioidomycosis included at least 2 of the following: fever, chills, night sweats, cough, dyspnea, chest pain, rash, arthralgia, myalgia, headache, anorexia, weight loss, and fatigue.

"Typical radiographic abnormalities" in coccidioidomycosis have been previously described (8). A radiograph or computed tomographic scan was considered abnormal if there were consolidations, nodular or patchy opacities, hilar and/or mediastinal adenopathy, pleural effusion, or a miliary pattern of disease.

For the purposes of this study and all related discussion in this article, "positive (coccidioidal) serology" is used when IgG reactivity was identified (with or without IgM) on at least 1 assay (EIA, CF, or ID) or when IgM was positive by ID. A serologic result with reactivity limited to IgM by EIA was not considered positive without confirmation by other positive serologic results.

Multiple methods were used for identifying coccidioidal antibodies, including EIA IgM and IgG (Meridian Bioscience test kit), ID for IgM (Meridian Bioscience test kit) and IgG (Gibson Laboratories test kit), and CF test (antigen obtained from the Coccidioidomycosis Serology Laboratory at the School of Medicine, University of California, Davis, Davis, CA). The EIA method used at our institution uses manual pipetting but an automatic plate washer.

At our institution, coccidioidal serology may be performed to assess the possibility of coccidioidomycosis in patients with typical symptoms or atypical febrile syndromes. In addition, patients who are undergoing evaluation for solid-organ or hematologic stem cell transplantation or who are being considered for institution of anti-tumor necrosis factor and other immune-modifying medications are screened serologically for the presence of coccidioidomycosis prior to the institution of immunosuppression. Generally, such patients do not manifest symptoms of infection at the time of screening.

To determine retrospectively whether our sample size had adequate power, we assumed that the proportions of confirmed, highly probable, or probable diagnosis were 0.4 for those who had serologic testing in the absence of symptoms and 0.8 for those who had serology for evaluation. If the type I error rate was 0.05, with at least 23 patients in each group, we would have at least 80% power to detect such a difference. We used simple descriptive statistics for analysis of the data obtained in the study using Microsoft Excel (Microsoft Corporation) or SAS 9.1 (SAS Institute). We used the Fisher exact test (Fisher's Exact Test Calculator for 2×2 Contingency Tables, Microsoft Computational Biology Web Tools [http://research.microsoft.com/en-us/um/redmond/projects/mscompbio/fisherexacttest/]) to compare proportions of discrete variables and the Wilcoxon rank sum test to compare the median of continuous variables. Using a 2-sided test, a P value of less than 0.05 was considered significant.

RESULTS

From 1 January 2004 through 31 December 2008, a total of 1,163 patients had positive coccidioidal serology or IgM-only EIA reactivity. Of these 1,163 patients, 1,117 patients (96%) had EIA serology performed, with multiple instances of testing for some patients, which resulted in 2,950 pairs of IgM and IgG by EIA.

One hundred two patients (9% of the 1,117 with EIA serology) had 141 EIA IgM$^+$/IgG$^-$ pairs (5% of 2,950 results). The distribution of the strength of diagnosis among these patients is summarized in Table 1. Among these patients, serology was performed

TABLE 1 Summary of characteristics of patients who had EIA IgM$^+$/IgG$^-$ serology

Patient group and strength of diagnosis	No. (%) of patients who were tested to:			
	Evaluate symptoms	Follow abnormal serology	Screen for coccidioidomycosis	Total
All study patients				
Confirmed	10 (17)	1 (8)	1 (4)	12 (12)
Highly probable	39 (65)	7 (54)	1 (4)	47 (46)
Probable	5 (8)	5 (38)	11 (39)	21 (21)
Possible	6 (10)	0	3 (11)	9 (9)
Unconfirmed	0	0	13 (45)	13 (13)
Total	60	13	29	102
Immunosuppressed patients only^a				
Confirmed	5 (45)	1 (50)	1 (11)	7 (32)
Highly probable	5 (45)	0	0	5 (23)
Probable	0	1 (50)	3 (33)	4 (18)
Possible	1 (9)	0	1 (11)	2 (9)
Unconfirmed	0	0	4 (44)	4 (18)
Total	11	2	9	22

^{a} Twenty-two patients were immunosuppressed and presented as a subset of the original group of 102 patients.

for the evaluation of coccidioidal infection-compatible symptoms ($n = 60$), to follow up on a prior serologic abnormality ($n = 13$), or for screening purposes ($n = 29$). The strength of diagnosis was determined for each patient and then delineated within each of the groups; results are summarized in Table 2. Of the 60 patients whose serology was performed to evaluate coccidioidal symptoms, 54 (90%) had confirmed, highly probable, or probable coccidioidal illness, and none was unconfirmed. Of the 29 patients whose serology was performed for screening purposes, 13 (45%) had confirmed, highly probable, or probable illness, and 13 (45%) had unconfirmed illness.

Differences in patient characteristics and findings of evaluation (whether performed for evaluation of symptoms or for screening) are compared in Table 2. Patients who had serology performed for evaluation of symptoms were significantly more likely to secure a diagnosis of pulmonary coccidioidomycosis ($P < 0.001$) with accompanying radiographic abnormalities ($P < 0.001$) than were patients who had serology performed for screening purposes. Likewise, patients who had serology performed for screening purposes were significantly more likely to have an unconfirmed serology ($P < 0.001$).

Among the 89 patients whose IgM-only reactive EIA serology was performed either for the evaluation of symptoms or for screening purposes, 12 (13%) subsequently underwent seroconversion; 5 of 29 (17%) patients whose serology was performed for screening later seroconverted to IgM$^+$/IgG$^+$, and for patients who had serology performed for symptoms and were found to be IgM$^+$/IgG$^-$, 7 of 60 (12%) seroconverted to IgM$^+$/IgG$^+$; the conversion rate between groups was not different ($P = 0.51$).

DISCUSSION

Coccidioidomycosis is a common infection within the area in which it is endemic, heralded by a spectrum of disease manifestations ranging from no symptoms (as seen in up to 60% of infected persons [9]) to a respiratory infection of variable severity. It is

TABLE 2 Comparison of characteristics in patients with EIA IgM$^+$/IgG$^-$ who had serology performed for evaluation of symptoms versus screening

Characteristic	No. (%) of patients who had serology for[a]:		
	Evaluation of symptoms ($n = 60$)	Screening ($n = 29$)	P value
Demographics			
Age, median (range), yr	55 (24–82)	52 (16–69)	0.11
White race	31 (52)	13 (45)	0.65
Strength of diagnosis: confirmed, highly probable, or probable	54 (90)	13 (45)	<0.001
Comorbid conditions			
Tobacco use	25 (42)	12 (41)	>0.99
Diabetes mellitus	8 (13)	10 (34)	0.03
Transplant	2 (3)	5 (17)	0.03
Pulmonary disease	6 (10)	1 (3)	0.42
Immunosuppression	11 (18)	9 (31)	0.19
HIV infection	0	0	>0.99
Characteristics of coccidioidomycosis			
Pulmonary infection	50 (83)	7 (24)	<0.001
Extrapulmonary infection	2 (3)	0	>0.99
Abnormal imaging	50 (83)	6 (21)	<0.001
Treated with antifungal agent	41 (68)	5 (17)	0.001

[a] Patients with prior abnormal results were omitted. Values are numbers (percentages) unless indicated otherwise.

typically characterized by one or more of the following symptoms: fever, chills, night sweats, headache, myalgia or arthralgia, cough, chest pain, shortness of breath, rash, and other manifestations (10). Primary pulmonary coccidioidomycosis accounts for 15% to 29% of community-acquired pneumonia (1, 11, 12) within the area of endemicity. When respiratory symptoms are present, many pulmonary coccidioidal infections are characterized by a dry, nonproductive cough. The dearth of easily expectorated sputum makes the diagnostic process more challenging, and serology is often used to secure the diagnosis. Serologic tests become positive at variable periods after the onset of illness (3) and remain positive for months to years but typically resolve after resolution of illness (2, 3). Because patients may have no symptoms at the time of illness, the infection may be unrecognized clinically yet serologically positive. Therefore, the interpretation of a positive serologic test may be problematic.

A number of test methods are available to detect coccidioidal IgM and IgG. The most common serologic tests currently in use are CF, ID, and EIA. Tests to detect complement-fixing IgG antibodies are well established, are performed in reference laboratories, and have the advantage of being quantitative (2). Optimal detection with CF peaks at approximately 1 to 2 months from the onset of symptomatic infection (3). The CF serologic titers fall with progressive convalescence (2). The detection of antibodies by ID is very specific and is often used to confirm a positive test by EIA; however, antibodies detected by ID may arise later in the course of infection than those detected by EIA (3). Coccidioidal serology detected by ID is also determined in reference laborato-

ries and may be reported as a quantitative result at certain laboratories (2).

The EIA is a useful test because it can be done locally and has demonstrated better sensitivity in early coccidioidal illness than other methods (3, 4). Two EIA kits are available commercially, without discernible differences in performance between them; both are easy in use, have a rapid turnaround time, and can be performed in local laboratories. One retrospective study demonstrated that 86% of patients with this test result had positive serologic findings by other methods (5), a finding echoed in the present study of 102 patients, in which 80 (78%) had either positive culture or histology or positive serologic findings by other methods. The demonstration of both IgM and IgG by EIA has been repeatedly shown to correlate well with other diagnostic assays in reference laboratories (6, 7, 13), but the finding of the IgM$^+$/IgG$^-$ remains problematic. Such problems with the IgM resolve when the serology is repeated (5), and seroconversion from isolated EIA IgM reactivity to EIA IgG reactivity occurs. Such seroconversion was seen in 20% of patients in a prior study (5). In the present study, seroconversion was observed in similar proportions of patients, whether initial serology had been performed for screening (5 of 29 patients [17%]; median seroconversion at 247 days) or for diagnostic purposes (7 of 60 patients [12%]; median seroconversion at 42 days). However, as illustrated by our findings, a minority of patients manifest such seroconversion, leaving health care practitioners with a diagnostic challenge in the case of IgM-only EIA reactivity.

The suboptimal clinical specificity of the EIA is explained partially by cross-reactivity with antigens to other fungi. Investigators have previously demonstrated problematic cross-reactivity with the EIA IgM serologic test in patients with known blastomycosis and other noncoccidioidal pulmonary illnesses (6). However, within the area in which coccidioidal infection is endemic, it is uncertain that this cross-reactivity accounts for more than a small percentage of laboratory studies, since *Blastomyces* organisms are not endemic in the same regions as *Coccidioides* species. On the other hand, the area of the southwestern United States in which coccidioidal infection is endemic is a desirable geographic location that many people from distant locales either visit or relocate to, so cross-reactivity to other endemic fungi cannot be quickly dismissed.

A previous study conducted within the area of endemicity by Kuberski et al. (7) demonstrated that a high percentage of isolated EIA IgM-reactive results were found in patients who were thought not to have coccidioidomycosis. Seventeen patients underwent testing by EIA for evaluation of symptoms and were found to have isolated EIA IgM reactivity. Samples were also sent to a reference laboratory for ID testing, and each patient's chart was retrospectively reviewed for an independent assessment of the presence of coccidioidomycosis (definitions were not provided). The isolated EIA IgM result was determined to be unconfirmed in 14 patients. The cohort consisted of 7 patients with clinical pneumonia, 5 with fever of unknown origin, and 4 with diagnoses not attributed to an infectious agent; the presence of compatible symptoms, radiographs, cultures, or serial serologic determinations was not presented for review. It is difficult to compare the results of the study by Kuberski et al. (7) with the results of the present study, since the former study did not take into account culture or pathology results and used the reference laboratory serology as the "gold standard" by which a diagnosis was made (potentially problematic

when the EIA may be seropositive earlier in the illness than ID studies [3]). Furthermore, early treatment of an acute infection may have blunted an IgG response (14). Finally, the former study had no evaluation for potential seroconversion.

In a comprehensive review of another center's experience with serologic diagnosis for coccidioidomycosis, Crum et al. (15) found that 18% of isolated EIA IgM reactivity was unconfirmed. Similar to the findings of the present study, Crum et al. (15) observed that the most unconfirmed results occurred in the year that more serologic tests were performed for surveillance purposes, rather than for the evaluation of symptoms. In the present study, 22 of 102 patients (22%) with isolated EIA IgM reactivity did not have a clear diagnosis of coccidioidomycosis that was confirmed by other serology or culture, and thus these may represent false reactivity. When we focused on patients who had serologic draws for screening purposes and not to evaluate symptoms, 16 of 29 patients (55%) had possible false-reactive results. An alternate explanation to a false-positive test result is that patients with coccidioidomycosis identified only by the newer EIA had fewer or milder clinical abnormalities than did patients in whom the infection was detected by older methods, as supported by a previous study (4).

A recent study indicated that the IgG serologic response may be blunted or absent in patients with primary coccidioidomycosis who received antifungal treatment within 2 weeks of symptom onset (14). Although 41 of 60 symptomatic patients (68%) in this study received antifungal medication, we did not find that the treated patients were less likely to experience a full serologic response than the untreated patients ($P = 0.07$). However, information regarding the timing of such treatment during the course of illness was not collected for this study.

A strength of our study is that a large number of tests were performed and a single laboratory conducted the serologic testing. In addition, it is not uncommon in our practice to simultaneously perform EIA, ID, and CF, providing other serologic results to assist with test interpretation. A previous study showed that all 3 methods were necessary for the best interpretation because the methods performed differently with different patient populations (3). Such ordering practices may not exist elsewhere on a routine basis. This study's origination from a tertiary referral center and retrospective design may also affect the generalizability of the results.

Although EIA is a sensitive and useful serologic test in early illness, the result of IgM-only EIA reactivity confers a diagnostic conundrum that may only be partially offset with culture, pathology, and repeated serology. The questions that remain present us with the ongoing need to better identify coccidioidal illness, especially early in the course of illness. A number of laboratories are evaluating novel assays to detect fungal antigens (rather than relying on a serologic result), and we welcome the opportunity to test such assays in the clinical arena. Until that time, we continue to study and better understand the tools that are currently available for clinical use.

In summary, the majority of patients in our study with isolated EIA IgM reactivity had coccidioidomycosis confirmable by other testing. Screening asymptomatic persons appeared to increase the rate of unconfirmable results but had a role in identifying active coccidioidal infections that required treatment during immuno-suppression. Interestingly, seroconversion from IgM-only EIA reactivity was similar between the symptomatic and screening populations. Serial reassessment of coccidioidal serology may clarify a result of EIA IgM$^+$/IgG$^-$ over time. Health care practitioners are advised to understand the advantages and disadvantages of the serologic methods used by their laboratory. We encourage future studies to identify and refine assays and methods that improve the ability to identify coccidioidomycosis in both symptomatic and asymptomatic patients.

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